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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

Date: March 30, 2000

MEMORANDUM

SUBJECT: Responses to Concerns Raised by Dr. Brian Dementi Regarding the HED Cancer Assessment of Malathion

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As you requested, I have reviewed the 14 areas of concern expressed in Dr. Dementi's memoranda. In summary, I found two issues that I recommend be considered by the Carcinogen Assessment Review Committee (CARC) and several areas that need editorial correction or clarification.

This memorandum responds to those concerns raised by Dr. Dementi about the cancer assessment of malathion. Dr. Dementi expressed these concerns in 22 memoranda dating from November 26, 1997 to February 9, 2000, all cited in and attached to the Carcinogen Assessment Review Committee Report of February 2, 2000 (called CARC Report in this memorandum), and in summary memoranda to John Carley, dated January 27, 2000, and February 3, 2000.

This memorandum clarifies the CARC position on the 14 issues raised by Dr. Dementi, but does not in itself revise the CARC position as stated in its report. Areas identified as needing further

evaluation or other clarification by the CARC will be considered at the April 6, 2000 CARC meeting. Any inconsistencies or errors identified in the CARC Report will also be corrected at that time.

References 21 and 22 identify items in the CARC Report that Dr. Dementi considered to be either factually incorrect, unclear or inconsistent. This memorandum addresses concerns expressed in reference 21. However, the response to reference 22, will be completed subsequent to the completion of this memorandum due to the large number of comments (about 50). These comments, as noted earlier, primarily involve errors or inconsistencies in the CARC Report. Any scientific issues should have been identified in the 14 areas of concern and responded to in this memorandum.

It should be noted that my references to female rat liver tumors are based on the data as it existed as of the February 22, 2000 CARC Report. Chiminova has recently submitted revised tumor incidences for these tumors based on a PWG evaluation. The new submission will be discussed at the CARC meeting currently scheduled for April 6, 2000. Therefore, my comments regarding female rat liver tumors may not apply if the new values are accepted. This applies primarily to items 8 and 14.

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1) Mouse liver tumors (ref. 1, 4, 5, 8, 16, 17, 18)
a) Dementi Summary: There was a positive liver tumorigenic response across all doses, i.e., no NOEL for males, and a positive response at the top two doses in females. The finding extending to the lowest dose in males, not unlike the liver tumorigenic response in the female rat in this respect, should be regarded as of particular concern.

Response:
These comments consider adenomas, carcinomas and the combined adenoma/carcinoma response in male mice.

- The combined response was driven by the adenomas.
- The carcinomas had no dose response and were not statistically significant either by pair-wise comparison or by trend.
- The two high doses (8000 and 16,000 ppm) (CARC report; Table 2) were considered to be positive (adenomas and combined) for a tumorigenic response. Although this was confounded by excessive toxicity at these doses, the tumor response was not "discounted."
- For adenomas at the lower two doses of 100 (15%) and 800 ppm (13%), there was no statistical significance by pair-wise comparison, no dose related increase, and the values were within the historical control range of 14 to 22%.[1] The tumor response was actually at the low end of the range.
- The concurrent controls were well below the historical control range (7% as compared to 14%). This supported the conclusion that, what could have been interpreted as a treatment-related increase of tumors at the two low doses, was actually due to an unusually low control incidence.
- When compared to the historical control data, the incidence of carcinomas at the low dose of 100 ppm (7%) was only slightly outside the range (0 to 6%), and the incidences of carcinomas at 800 ppm (5%) and 8000 ppm (4%) were within the historical control range. In the five historical control studies, the incidences of liver carcinomas were: 0 in 3 studies; 1 mouse in one study (2.2%); and 3 mice in an another study (6.4%).
- Tumors (adenomas) occurred in the control animals.

The tumor incidence in female rats at 100 and 500 ppm, was considered to be suggestive evidence of carcinogenicity and could not be discounted for the following reasons:

- Although the incidences were not statistically significant, they were above the historical control mean.
- There were no tumors in the concurrent control group.
- This tumor has a low historical control incidence in female rats.
- There was a positive response in at a non-toxic dose (6000 ppm).

Therefore the CARC was concern about the low dose response in the female rats.

There are several differences in the low dose response between the male mice (noted above) and the female rats. 1) This is a common tumor in male mice while it is uncommon in female rats; 2) the incidences in mice at the low doses were at the low end of the historical control range while

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[1] combined values and the means were not available

they were above the historical control mean; 3) There were tumors in the controls male mice (although at an unusually low incidence), but no tumors at all in the female rat control group.

Although the CARC agreed with Dr. Dementi's comment that there was a positive response in the two high doses in both male and female mice, for the reasons delineated above; the CARC did not consider there to be a tumorigenic response at the two low doses in male mice. In addition, although the CARC considered the effects at the high doses to be positive in males, they also considered these doses to be excessive due to marked cholinesterase inhibition. There was also decreased absolute body weights ranging from 9.7 to 20 % depending on sex and dose. Based on this toxicity, the CARC felt that positive tumor data at the two high doses, when considered with the rest of the data base was supportive of (rather than evidence for) the qualitative determination of malathion as a "likely human carcinogen." The data at these high doses was not discarded.

Table 2. Male Mice: PWG Re-read, 1998 - Liver Tumor Rates[+] and Exact Trend and Fisher's Exact Test Results

Tumor Type	0 ppm	100 ppm	800 ppm	8000 ppm	16,000 ppm
Adenomas	4/54	8[a] /54	7/55	**14[a] /55**	**49[a] /51**
%	7	15	13	**25**	**96**
p=	**0.000****	0.180	0.274	**0.0103***	**0.000****
Carcinomas	0/54	4/54	2[b] /55	2/55	0/51
%	0	7	5	4	0
p=	0.128	0.059	0.252	0.252	1.0
Combined	4/54	10[c] /54	9/55	**15[d] /55**	**49/51**
%	7	19	16	**27**	**96**
p==	**0.000****	0.075	0.125	**0.006****	**0.000****

[+] =Number of tumor bearing animals/Number of animals examined, excluding those that died before week 54. Also excludes week 53 interim sacrifice animals (Statistical Analysis, Brunsman, 2/16/99).

[a] First liver adenoma observed at week 53, dose 16,000 ppm, in an interim sacrifice animals. Subsequent liver adenomas observed at week 79, simultaneously in the 100, 8000 and 16,000 ppm dose groups, in terminal sacrifice animals.

[b] First liver carcinoma observed at week 65, dose 800 ppm.

[c] Two animals in the 100 ppm dose group had both an adenoma and a carcinoma.

[d] One animal in the 8000 ppm dose group had both an adenoma and a carcinoma.

Note: Interim sacrifice animals are not included in this analysis. One male in the 16,000 ppm dose group of the interim sacrifice group had a liver adenoma.
Significance of trend denoted at control.
Significance of pair-wise comparisons with control denoted at dose level.
If *, then p <0.05; If **, then p <0.01

1) Mouse liver tumors (continued)
b) Dementi Summary: The CARC should not leave unexplained, the more remarkable liver tumorigenic responses, particularly in females, that were observed in the more recent mouse study as opposed to those in the earlier NCI study. This is of particular concern since the new study was designed to replicate at the top two doses.

Response:
As stated in the CARC Report:

> "In the 1978 NCI study with B6C3F1 mice, liver tumors (11 carcinomas and 6 adenomas) were seen in 17 of 55 male mice at the highest dose tested (16,000 ppm); there was no carcinogenic response in female mice. Also in the NCI study, among females, the combined adenomas/carcinomas incidences were 0% at 8000 ppm and 4% at 16,000 ppm in contrast to the present study where the tumor incidences in females were 19% at 8000 ppm and 84% at 16,000 ppm. The Committee noted that the tumor responses in the present study at the same dose levels were more pronounced than those seen in the NCI study."

Other than making the observation that the more recent mouse study has a more pronounced response, the CARC was unable to make any further observations. Given the information the Committee had about both studies, anything further would be speculation and would not add to the risk assessment process.

2) Thyroid c-cell tumorigenic response in the male rat (ref. 10, 17)
Dementi Summary: This finding is positive among male rats across the 0-500 ppm dose range, and cannot be discounted as CARC has done by findings at higher "excessive" doses, lest the study be considered unacceptable for evaluation of this tumorigenic response. Findings at low doses should be of particular concern and discounted only by the most persuasive forms of evidence.

Response:
(CARC report; Table 10a) Following discussion with the consulting veterinary pathologist, the incidences of combined thyroid c-cell tumors were determined to be the most appropriate tumor values for the final evaluation due to the difficulty in distinguishing the individual tumor types (i.e., adenomas vs. carcinomas)[2]. It is true that there is statistical significance by pair-wise comparison for thyroid c-cell carcinomas at the 500 ppm (both with and with out considering the 2 high doses) (2%, 4%, 13%**, 5%, 0%, for controls to high dose). The CARC did consider the possibility that the excessive mortality in males at the top doses (74% at 6000 ppm and 100% at 12,000 ppm) may have compromised the expression of this tumor at these (higher) doses. However, the Committee noted that at 6000 ppm there were still 43 rats considered to be at risk (alive after the first occurrence of carcinoma) which was considered to be an adequate number for evaluation. Therefore, there was no dose response and the increase at 500 ppm was considered to be due to variation rather than malathion. For the combined tumors, there was no statistically significant trend, pair-wise significance, or dose-response at any dose level, either when all dose groups were included or when the top two doses were excluded from the analyses. Additionally, there was no evidence of malathion induced thyroid toxicity in the database and there were no supportive pre (non) neoplastic lesions in the thyroid glands of male or female rats. Therefore, the Committee did not agree with Dr. Dementi, and considered that the thyroid c-cell tumors were not attributable to treatment.

[2] Also see Reference: McConnell, E. E., Solleveld, H. A., Swenberg, J. A. and Boorman, G. A. (1986) Guidelines for Combining Neoplasms for Evaluation of Rodent Carcinogenesis Studies. JNCI, 76, pp. 283-289.

Table 10a. Male Rat: Thyroid C-Cell Tumor Rates[+] and Peto's Prevalence Test Results Including All Dose Groups

Tumor Type	0 ppm	100/50 ppm	500 ppm	6000 ppm	12,000 ppm
Adenomas p=	13/53 (25%) 0.326	14/54 (26%) 0.461	10/50 (20%) -	6/50 (12%) -	4[a]/35 (11%) 0.242
Carcinomas p=	1/51 (2%) 0.556	2/50 (4%) 0.310	6[b]/45(13%) **0.012***	2/43 (5%) 0.178	0/9 (0%) -
Combined p=	14/53 (25%) 0.430	16/54 (30%) 0.389	14[c]/50(28%) 0.403	8/50 (16%) -	4/35 (11%) 0.242

[+] Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before observation of the first tumor (Statistical Analysis, Brunsman, 5/3/99).

[a] First thyroid c-cell adenoma observed at week 81, dose 12,000 ppm.

[b] First thyroid c-cell carcinoma observed at week 90, dose 500 ppm.

[c] Two animals in the 500 ppm had both an adenoma and a carcinoma.

3) Thyroid follicular cell tumors (ref. 16, 17, 18)
Dementi Summary: "The competing toxicity and increased mortality among male rats at 6000 ppm and 12,000 ppm (dose levels considered as excessive by CARC) may have *dampened or compromised* full expression of a tumorigenic response at these higher doses already evident in the existing data set, i.e. a positive dose trend ($p = 0.035$) and a nearly positive ($p = 0.077$) pair-wise comparison for the 6000 ppm dose group. I challenge, therefore, CARC's conclusion that the study can be accepted as a negative study for this tumorigenic response. In my view (not stated as such previously, though evident in the reasoning) this tumorigenic response should be viewed as suggestive evidence of carcinogenicity that cannot be discounted because of the unacceptability of the study in male rats at the high dose levels, which CARC itself has called excessive. This is a difficult interpretation which I feel merits an external review."

Response:
(CARC Report; Table 9) The Committee concluded that the thyroid follicular cell tumors were not treatment-related since there was neither a pair-wise significance nor a dose-response relationship for any thyroid follicular cell tumor type (i.e., adenomas, carcinomas or combined adenomas/carcinomas); only a trend was seen for the combined tumors. The argument presented by Dr. Dementi that "competing toxicity ... may have dampened or compromised full expression of a tumorigenic response ..." is speculative and in this case, the Committee felt that it was inappropriate to speculate what would have happened if mortality wasn't so high at the two high doses.

In addition, although the CARC considered the 6000 and 12,000 ppm dietary concentrations to be excessive for male rats based on mortality and cholinesterase inhibition in all three compartments, the 500 ppm concentration was considered **adequate** (not too low—inadequate) to evaluate carcinogenicity. (For additional details see item 13.) Therefore, the 500 ppm dose was considered to be appropriate for use when evaluating the carcinogenic potential of malathion in the male rat—without requiring any intermediate doses or a new study.

It should be noted that **I recommend** revising the executive summary and weight of evidence sections of the CARC Report to include a statement consistent with what was said for the male rat liver tumors: "**Although there was no evidence of these tumors in rats at any dose level, the potential for tumor induction may have been compromised by competing toxicity, particularly at 6000 ppm and 12000 ppm, where mortality was 74% and 100%, respectively. There is, however, no evidence to either support or refute this supposition.**" This statement would acknowledge Dr. Dementi's assertion. However, the CARC did not think that it would be appropriate to suggest that there would have been more tumors if a dose in between 500 and 6000 ppm was tested.

Table 9. Male Rat: Thyroid Follicular Cell Tumor Rates[+] and Peto's Prevalence Test Results.

Tumor Type	0 ppm	100/50 ppm	500 ppm	6000 ppm	12,000 ppm
Adenomas	2/55	1/54	1/51	4/51	4[a]/43
(%)	4	2	2	8	9
p=	0.063	-	-	0.150	0.378
Carcinomas	0/42	0/45	2/41	2[b] /26	0/0
(%)	0	0	5	8	0
p=	0.196	-	0.085	0.162	-
Combined	2/55	1/54	3/51	6/51	4/43
(%)	4	2	6	12	9
p=	**0.035***	-	0.321	0.077	0.160

[+] =Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before observation of the first tumor (Statistical Analysis, Brunsman, 7/16/97).
[a] First thyroid follicular cell adenoma observed at week 76, dose 12,000 ppm.
[b] First thyroid follicular cell carcinoma observed at week 100, dose 6000 ppm.

Note: Interim sacrifice and accidental death animals were not included in this analysis. There were no thyroid follicular cell tumors in any of the interim sacrifice or accidental death animals.

Significance of trend denoted at control.
Significance of pair-wise comparisons with control denoted at dose level.
If *, then $p < 0.05$; If **, then $p < 0.01$

4) Leukemia in the rat: Interpretation of evidence under OSTP (1985)'s definition of carcinogen (ref. 7, 9, 17)

Dementi Summary: "Evidence of a dose related increased incidence of mortality attributed to leukemia ***among male rats diagnosed with leukemia*** constitutes positive evidence of carcinogenicity under the second aspect of OSTP's definition of carcinogen, namely, '... or significantly decreases the time it takes a naturally occurring (spontaneous) tumor to develop relative to an appropriate background or control group. Either phenomenon is said to represent the effects of a carcinogen.' (pp. 10410-10415). I contend the dose-related increased mortality (where mortality itself indicates a more advanced stage) is evidence of a dose-related increased rate of development of leukemia. It could be argued that rats harboring leukemia are simply more susceptible to early death due to the increasing secondary toxicologic burden of the test material, but to confirm that possibility and to discount the possibility of a direct compound effect in development of the response, the mechanism would need to be established. I am not aware CARC has provided a rational response to this issue."

Dr Dementi elaborated on this in Ref. 9, noting that leukemia and nephropathy are the primary causes of death in this study. "The number of male rats among 55 rats per group diagnosed with leukemia (death due to leukemia) were 23(7), 16(7), 24(14), 18(13) and 1(1) for the control, 100/50, 500, 6000 and 12,000 ppm groups, respectively. Hence, among rats diagnosed with leukemia, the percentages dying with leukemia were: 7/23 (30%), 7/16 (44%), 14/24 (58%), 13/18 (72%) and 1/1 (100%), in the same respective order." Dr. Dementi further observed that the rate of rats dying due to nephropathy also increased with dose to 47 out of 55 at the high dose. Therefore, he proposed that the decreased expression of leukemia, at least the high dose, is due to competing toxicity from nephropathy.

Response:

(CARC Report; Table 16) The CARC examined this endpoint at several meetings (OCT-15, 1997, FEB-24-1999, JUN-23-1999). The Committee first evaluated the mononuclear cell leukemia (MCL) at the October 15, 1997 meeting, and concluded that the occurrence of this tumor type in female rats was not attributable to treatment because there was no statistical significance at any dose level and the incidences were within in the historical control range of the testing laboratory (15 to 36%). Subsequently, at the February 24, 1999 meeting, the Committee determined that additional statistical analysis using Peto's prevalence test was needed to more accurately evaluate the significance of this tumor type in male rats. Results of this analysis—presented below (CARC Report; Table 16)—were evaluated at the June 23, 1999 CARC meeting. The Committee concluded at that time, that MCL in male and female rats was not treatment related based on: 1) the lack of statistical significance at any dose level, 2) absence of a dose-response relationship, and 3) the incidences were within the historical control range of the testing laboratory (15 to 36%). Additionally, the CARC Report noted that MCL was not seen in three strains of rats: the Osborne-Mendel (1978 NCI-malathion); Sprague-Dawley (1980-FDRL-malathion); and F344 (1979, NCI-malaoxon and the 1996 malaoxon studies). **I recommend** that the following disclaimer be added to this section of the CARC report, "However, the results of the old studies should be used with caution to support or refute any results due to inherent problems in these studies."

Based on review of the minutes for meeting dated June 23, 1999 (printout from the white board), it appears that the issue of "**percent of leukemic animals dying from MCL**," while in the background package given to the CARC members for review, was inadvertently not discussed at the meeting.

I recommend that the CARC reconsider this subject, based on the information presented by Dr. Dementi (Table A, taken from the DER and ref. 9). It appears that there may be evidence of increased severity—as evidenced by the increased percentage of leukemic animals dying due to MCL—with the exception of the high dose. While there is no supporting evidence that competing toxicity from nephropathy is responsible for the decrease in MCL at the high dose, the expression of MCL is extremely low considering that the number of animals considered to be at risk is 52 (alive at time of first occurrence of MCL). This lesion is considered fatal in the more advanced stages of severity.

This may be consistent with the SAP comment (below) that the severity could be increased due to chemical exposure. Since severity was not staged by the study pathologist, the CARC needs to determine if it is supported scientifically to use the incidence of these tumors as the cause of death (determined by the study pathologist) as an indicator of the increased severity at the later stages.

The Scientific Advisory Panel report, "A Set of Scientific Issues Being Considered by the Agency in Connection with DDVP (Dichlorvos) Risk Issues" addressed the use of MCL in cancer risk assessment. "There is an emerging view based on cumulative experience by some toxicologic pathologists that mononuclear cell leukemia in the Fischer rat may be a unique type of cancer and not induced *de novo* by compound administration....There is compelling evidence to disregard MCL, in the Fischer rat. MCL is one of the most common background tumor types in this strain, and has been referred to as Fischer rat leukemia. Other rat strains and mice do not develop MCL, and there is no human correlate to this disease. Additionally, chemically-related increases in MCL exhibit advanced severity grades for this lesion in treated rats compared to controls."

The relevance of this increase—if it is determined to be real—to human risk assessment is questionable according to the SAP and as presented in a new review of MCL (ref. 26). The DDVP CARC Report #6 (1-MAR-2000) indicated that: 1) MCL is common in the Fischer rat and, in the males ..., 2) The tumor type does seem to be found mainly in this Fischer strain and does not appear to be similar to leukemia in humans (adults or children). The CARC concluded that, "while all of this information somewhat lessened our concern, the MCL could not be totally dismissed as not being relevant to humans."

Table 16. Mononuclear Cell Tumor Rates[+] and Peto's Prevalence Test Results.

Tumor Type	0 ppm	100/ 50 ppm	500 ppm	6000 ppm	12,000 ppm
Male	23/55	16/55	24/55	17/53	1[a]/52
(%)	42	29	44	32	2
p=	-	-	0.463	-	-
Female	9/55	**18/55**	15/55	13/54	10[b]/55
(%)	16	**33**	27	24	18
p=	0.917	**0.025***	0.059	0.181	0.670

[+] =Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before observation of the first tumor (Statistical Analysis, Brunsman, 7/16/97 & 5/3/99).

[a] First mononuclear cell leukemia observed in a males at week 64, dose 12,000 ppm.

[b] First mononuclear cell leukemia observed in a female at week 47, dose 12,000 ppm.

Note: Interim sacrifice animals are not included in this analysis. There were no mononuclear cell leukemia in any of the interim sacrifice animals.

Significance of trend denoted at control.

Significance of pair-wise comparisons with control denoted at dose level.

If *, then $p < 0.05$; If **, then $p < 0.01$

Table A. Male rats (Fischer 344) with MCL that died from MCL

DOSE	control	100/50 ppm	500 ppm	6000 ppm	12,000 ppm
MCL as cause of death/# with MCL	7/23	7/16	14/24	13/18	1/1
% animals with MCL, dying from MCL	30	44	58	72	100

Table taken from the DER (MRID 43942901)

MCL - mononuclear cell leukemia

5) Interstitial cell testicular tumor in the rat (ref. 8, 11, 16, 17)

Dementi Summary: "Statistical significance of this tumorigenic response was positive across all four doses as presented in the study report, and was positive across the top three doses as analyzed by the Peto test within HED. I accept these assessments as showing a dosing related higher incidence than expected of this tumorigenic response, and hence, as a positive carcinogenic effect by recognized definitions of a carcinogen. In my view, the Peto test, as required by the CARC, was conducted in the prescribed manner by HED's statistician, and was positive. I am not satisfied with CARC's rationale (absent mechanistic data) for discounting this response, and would desire another expert opinion."

Response:

(CARC Report; Table 15) At the October 8, 1997 CARC meeting, the Committee determined that male rats had a significant increasing trend, and a significant difference in the pair-wise comparison of the 12,000 ppm dose group with the controls for the interstitial cell tumor, both at $p < 0.01$—using the Peto's Prevalence Analyses protocol. There were also significant differences in the pair-wise comparisons of the 500 ppm and 6000 ppm dose groups with the controls for this tumor type, both at $p < 0.05$. Statistical analyses of this tumor in the study report indicated that the increases in testicular tumors were statistically significant at all dose levels. Statistical analysis by HED obtained essentially the same results, except for the low dose group which did not show pair-wise significance. However, statistical evaluations should not be considered to be the final word without any consideration of the biological relevance of the data. For this tumor type, the historical spontaneous occurrence often approaches 100% by the end of a study. Therefore, in spite of the above statistical evidence, the Committee concluded—and I agree—that, contrary to Dr. Dementi's opinion, the testicular tumors should not be considered treatment related since: 1) this non-lethal tumor was observed in nearly 100% of male rats including controls; 2) the apparent statistical significance of the tumor incidence at 6000 and 12,000 ppm groups [***Note***: both doses were determined to be excessive in males] could be attributed to the high mortality at these doses—resulting in earlier observation of the tumor—and significance was considered to be an artifact of the Peto's Prevalence Analyses protocol; 3) sufficient data were not available to determine if there was a decrease in the latency period [i.e., there was no serial sacrifice to determine latency]; and 4) this tumor type is not useful in overall evaluation since its occurrence is similar at all dose levels.

Table 15. Male Rat: Testes Interstitial Cell Tumor Rates[+] and Peto's Prevalence Test Results (p values)

Tumor Type	0 ppm	100/ 50 ppm	500 ppm	6000 ppm	12,000 ppm
Interstitial cell tumor	52/55	52/55	53/55	52/53	53 [a] /54
(%)	95	95	96	98	98
p=	**0.000****	-	**0.037***	**0.032***	**0.004****

[+] = Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before observation of the first tumor (Statistical Analysis, Brunsman, 7/16/1997).
[a] First testicular tumor observed at week 54, dose 0 ppm, in a 54-week interim sacrifice animal. First testicular tumor not in an interim sacrifice or accidental death animal observed at week 64, dose 12,000 ppm.

Note: Interim sacrifice and accidental death animals are not included in this analysis. Two animals in the 0 ppm dose group and five animals in the 12,000 ppm dose group of the 54-week interim sacrifice group had this tumor. Two accidental death animals in the 6000 ppm dose group had this tumor.

Significance of trend denoted at control.

Significance of pair-wise comparisons with control denoted at dose level.

If *, then $p < 0.05$; If **, then $p < 0.01$

6) Rat nasal tissue histopathology and tumorigenic response in the rat (ref. 12, 13, 14 16, 17, 19, 20)

Dementi Summary: "I accept as evidence of carcinogenicity all **four** extremely rare nasal tumors, two in males and two in females, at the top two dose levels. CARC discounts the findings in males, as I understand, because dosing was excessive, but again, as with certain other tumor types, to the extent tumorigenic findings are discounted in high dose groups, the study in my view is unacceptable in males. The issue is complicated by evidence of nasal histopathology in the long term combined chronic toxicity/carcinogenicity studies in the F344 rat for both malathion and malaoxon, and in the dose range-finding and subchronic inhalation studies of malathion in the rat. While a new inhalation study is being required, I am not satisfied that CARC has an adequate interim handle on risks posed with respect to the nasal mucosa, particularly by the inhalation route of exposure. Nasal tissue vulnerability is an important and unresolved issue at this time."

Response:

I have identified several inconsistencies in the CARC Report regarding the male nasal tumors and their contribution to the weight of the evidence. These need to be corrected when the report is revised following the April CARC meeting. The CARC report should be corrected to consistently say that, 1) the nasal tumor in the high dose males is supportive (but not strong evidence by itself) evidence, 2) the biological significance of the olfactory epithelial tumor is unknown since it is from a different cell of origin and these types of tumor (esthesioneural epithelial neoplasms) should not be combined with other tumors of the respiratory nasal cavity[3].

Based on the data currently in house, the CARC can not develop (as requested by Dr. Dementi) an "interim handle on risks posed with respect to the nasal mucosa, particularly by the inhalation route of exposure." It is hoped that the required 90 day inhalation study will shed light on this issue. In the absence of any other information, The CARC stated, "In accordance with the EPA *Proposed Guidelines for Carcinogen Risk Assessment* (April 10, 1996) [ref. 25], the Committee classified malathion as a **"likely human carcinogen** by all routes of exposure." This includes the inhalation route. Therefore, in the absence of data, I feel that the CARC is taking the most conservative approach by considering there to be a potential carcinogenic risk by exposure from the inhalation route.

(CARC Report; Table 8) The CARC agrees with Dr. Dementi that the nasal tumors (respiratory adenoma) in the female rats are evidence of carcinogenicity. "**The Committee ... concluded that there is evidence of carcinogenicity for malathion in female rats** ... which manifested as ... tumors of the nasal mucosa at 6000 ppm, although nasal tumors were also seen at 12,000 ppm (a dose considered to be excessive)." The statement "(but not in males)," that was in the previous sentence in the report, will be removed (as noted above) since it is inconsistent with the remainder of the CARC Report. The CARC did not discount the tumor at the female high dose,

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[3] Also see Reference: McConnell, E. E., Solleveld, H. A., Swenberg, J. A. and Boorman, G. A. (1986) Guidelines for Combining Neoplasms for Evaluation of Rodent Carcinogenesis Studies. JNCI, 76, pp. 283-289.

but felt that more weight should be placed on tumors that occurred at non excessive doses.

There are two different types of tumors in the male—an **adenoma of the olfactory epithelium** at 6000 ppm and an **adenoma of the respiratory epithelium** at 12,000 ppm compared to zero for each in the controls. The CARC considered that the adenoma of the respiratory epithelium added to the concern even though it was at an excessively toxic dose. As noted earlier, these two tumor types should not be combined. Therefore, it can not be determined whether the olfactory epithelial tumor increases our concern or not for nasal tumors in the rat.

Table 8. Neoplastic Findings of the Nasal/Oral Tissues in Rats

TUMOR TYPE	Dose (ppm)				
	0	**100/50**	**500**	**6000**	**12,000**
MALES (No. Examined: 90/dose[a])					
Nasal Olfactory Epithelium **Adenoma**	0	0	0	**1**	0
Nasal Respiratory Epithelium **Adenoma**	0	0	0	0	**1**
Palate, Squamous Cell papilloma	0	1	0	0	0
FEMALES (No. Examined: 90/dose[a])					
Nasal Respiratory Epithelium **Adenoma**	0	0	0	**1**	**1**
Tooth, Alveolus, Squamous Cell Carcinoma	0	1	0	0	0
Palate, Squamous Cell Papilloma	0	0	0	1	0
Palate, Squamous Cell Carcinoma	0	0	0	0	1

[a] This is uncensored data. There were only 55 rats/sex/dose in the 2 year portion of this study, the remainder were sacrificed at 3, 6 or 12 months.

7) Oral cavity assessment for tumorigenic response: Its adequacy and CARC's conclusion regarding squamous cell tumorigenic response (ref. 13, 14, 15, 17, 19, 20)
Dementi Summary: "I contend the **four** extremely rare squamous cell tumors (three in females, one in males) appearing in oral mucosal tissues in the malathion combined chronic toxicity/carcinogenicity study in the rat cannot be discounted as evidence of carcinogenicity. Furthermore, as these tumors were identified in but a partial and inadequate assessment of oral cavity histopathology, there is a greater incumbency to accept these as real until an adequate histopathology assessment of the entire oral cavity tissues has been performed. I have suggested this need for additional histopathology to CARC, and am concerned the registrant did not of his own volition follow-up with a complete oral cavity histopathology assessment once these tumors were found. CARC discounted the oral tumors at one meeting on the grounds these tumors are not as rare as the nasal tumors. However, subsequent follow-up information, in my opinion, demonstrates the squamous cell tumors to be essentially as rare as the nasal tissues in various data bases. I am not aware CARC has responded to the more recent information. I have not been availed of the latest, or final, CARC report."

In ref. 14, Dr, Dementi noted, "In consideration of the fact that the four nasal tumors were considered treatment-related while the four oral tumors were not at the June 23 CARC meeting, toward the end of the meeting a CARC member sought an explanation for this voting disparity. The CARC response was clear, the nasal tumors are very rare, historically, but the oral tumors are not. Yet, this subsequent and closer scrutiny of the NTP data base indicates that squamous cell tumors of the palate are as rare as the nasal tumors. The rationale for the difference in vote does not exist."

Response:
(CARC Report; Table 8, above) The CARC Report concluded that, "Palate tumors were observed at 100/50 ppm (a squamous cell papilloma in 1/90 males), at 6000 ppm (a squamous cell papilloma in 1/90 females) and at 12,000 ppm (a squamous cell carcinoma in 1/90 females). These tumors were not attributed to malathion treatment due to lack of statistical significance, and absence of a dose-response in either sex." The support for this was that they were not as rare as the nasal tumors.

I recommend that this issue be reexamined by the CARC. This is based on the information presented by Dr. Dementi, particularly the new historical control discussion indicating that they are rare (ref. 13). Dr. Dementi makes a valid point that, once the difference in historical controls goes away, the oral tumors have the same pattern of occurrence as the nasal tumors and should be evaluated with the same criteria. Also of concern is the squamous cell tumor of the tooth (alveolus) in a low dose female. This is also an oral squamous cell tumor and should be combined with the other oral squamous cell tumors (phone conversation with Dr. Brenneke, FEB-29-2000, 8:50 AM) resulting in single tumors in the 100/50, 6000 and 12,000 ppm female groups. Therefore, there may be an additional concern for this tumor type in females since there are two groups with tumors in the absence of excess toxicity. In the males however, there is only 1 tumor, and that occurs in the low dose where there are no other oral tumors. Therefore, I feel that it is difficult to attribute any biological significance to the occurrence of a single tumor occurring only at the low dose.

I have some concern that the historical control values as presented by Dr. Dementi are misleading. All squamous cell tumors of the oral cavity should be considered together. The historical control data as presented in ref. 20, are for tumors of the palate and for tumors of the tooth, independently. The appropriate historical control data should include all animals bearing squamous cell tumors of the oral cavity. The values presented in ref. 14 from the NTP data base, did support Dr. Dementi's contention that these are rare tumors in rats. There were very few squamous cell carcinomas (0/901) and papillomas (2/901) of the oral mucosa (including the palate) in females. Values for male rats were similarly low, 1/904 and 2/901 for carcinomas and papillomas, respectively.

Dr. Dementi also expressed concern that the oral cavity was not routinely examined histologically. In his memorandum to Patricia Moe (SRRD) (ref. 19) he says that, "Dr. Bolte indicated that all cavities, oral cavity included, received postmortem examinations for macroscopic abnormalities, and that the tissues associated with the oral cavity included the lips, gingiva, teeth, buccal mucosa, tongue and hard palate." "Specifically, Dr. Bolte provided assurances that oral cavity tissues in question were examined macroscopically, but he advised that the oral cavity is not a protocol tissue." Therefore, negative findings were not reported and the tissue did not routinely undergo histologic examination. There were no macroscopic lesions observed in the oral cavity, indicating that the oral tumors were all microscopic in size. Oral tissue was only examined incidentally in nasal sections. We do not know how many had slides with incidental negative oral tissue present since this was not reported. As a result, the actual incidences of these tumors in the study may be underestimated. Therefore, **I recommend that the Committee consider the advisability** of asking for a routine microscopic examination of the oral cavity, if it appears it could have an impact on the ultimate weight of evidence and cancer classification.

8) Tumorigenicity (several end points) in low dose groups [Concerted evidence of]. For example, are the low dose hepatocellular tumorigenic responses in the mouse and rat mutually supportive? (ref. 1, 15, 16, 17)

Dementi Summary: "I have expressed concern over certain tumorigenic responses that appear to extend into the low dose range, incorporating in certain cases even the lowest dose, absent a NOEL (e.g. male mouse liver tumors, female rat liver tumors, leukemia in male rats as defined above, extremely rare oral squamous cell tumors, possibly testicular tumors). My concern is whether collectively these speak more strongly of a low dose biological effect, than any standing alone, and whether CARC has adequately addressed this possibility in its assessment."

Response (see last paragraph, page 2):

In response to Dr. Dementi's concern regarding low dose tumors, it should be noted that the CARC Report did not consider there to be a low dose response in any of the tumors, with the exception of liver tumors in the female rat. The issue of oral squamous cell tumors occurring at the low dose in female rats will be revisited. The CARC does consider that the quantitative risk assessment using the female rat takes into account the increase in tumors observed at all doses, including the low dose. CARC Report already expressed concern for one (this may be changed to two) tumor type occurring at the low dose. The CARC considered this in the weight of evidence, and classified malathion as a "likely human carcinogen." Dr. Dementi questions whether the CARC has adequately addressed the possibility that collectively the occurrence of low dose tumors "speak more strongly of a low dose biological effect, than any standing alone. ..." I feel that the narrative that accompanies the cancer classification, adequately describes the tumors of concern, noting factors (such as occurrence at either low or excessive doses) which increase or lessen this concern. Therefore, no additional evaluation is required, with the exception of oral tumors in the female rat and MCL in the male rat and how they affect the weight of evidence, if at all.

9) Decisions to discount dose levels as excessive for carcinogenicity assessment based on cholinesterase inhibition (ref. 1, 8, 12, 16)

Dementi Summary: "Inherent in such review would be the precedent for the decision, existence of guidelines, which forms of the enzyme must be inhibited and by how much, and so on. I do not accept the view that cholinesterase inhibition (absent any guidelines or rationale) alone, absent cholinergic clinical signs, can be cited as adequate rationale to discount a dose level in question as excessive, and in so doing discount remarkable tumorigenic findings observed at that dose level. In my view, inadequate rationale has been provided by CARC to justify dismissal of dose levels as excessive, and in so doing precluding testing at high doses (MTD) called for in cancer bioassays."

Response:

I identified an error by omission in the CARC Report executive summary. The report stated "The Committee concluded that in mice, the 800 ppm dose level was adequate to assess the carcinogenic potential of malathion, however, the 8000 and 16,000 ppm doses were excessive based on severe plasma (90 to 95%) and red blood cell (92 to 96%) and marked brain (20 to 43%) cholinesterase inhibition in both sexes." It didn't mention that at these doses, there was also a marked decrease in absolute body weight (throughout the study). This is discussed in the body of the CARC Report and will be added to the executive summary in the revised report.

The CARC considers cholinergic inhibition in conjunction with the rest of the data base for a particular study and chemical. This includes: how many compartments (with particular attention to brain cholinesterase) are effected, the magnitude of response, the presence of clinical signs, changes in body weight and food consumption, mortality as well as the presence of cholinergic signs.

In the Fischer 344 rat malathion cancer study, the doses of 500 ppm in males and 6000 ppm in females were considered adequate to assess the carcinogenic potential of malathion; but 6000 ppm in males was excessive due to increased mortality (74%); and the 12,000 ppm was excessive in both sexes based on the severe inhibition of plasma (89%), red blood cell (52%) and brain (67%) cholinesterase activity in females and increased mortality in males (100%) and females (64%) at this dose. In this case, not only was there evidence of cholinesterase inhibition in all three compartments, there was also increased mortality. This was also true for the rat malaoxon study where the dose level of 1000 ppm was considered adequate to assess the carcinogenic potential of malaoxon, but the 2000 ppm dose was excessive due to increased mortality (53% in males and 49% in females) and severe inhibition of plasma (83-96%), red blood cell (54-66%) and brain (11-78%) cholinesterase activity.

In the mouse malathion cancer study, the 800 ppm dose level was considered adequate to assess the carcinogenic potential of malathion, however, the 8000 and 16,000 ppm doses were considered excessive based on severe plasma (90 to 95%),severe red blood cell (92 to 96%) and marked brain (20 to 43%) cholinesterase inhibition in both sexes. All three compartments were affected and there was almost 100 % inhibition in the first two. The NOAEL for plasma and RBC cholinesterase inhibition in the mouse was 100 ppm, and that for brain cholinesterase inhibition was 800 ppm for both sexes—substantially lower doses than those considered

excessive. There was also decreased absolute body weights at 8000 and 16,000 ppm in both sexes, ranging from 14.3-20.0% in males and 9.7-16.1% in females throughout the entire duration of the study. The final body weights for the 8000 and 16,000 ppm groups were between 3 and 7 grams less than controls. Although there was neither mortality or clinical cholinergic signs of toxicity in the mouse, the presence of marked cholinesterase inhibition and decreased absolute body weights at 8000 and 16,000 ppm in both sexes is supporting evidence of excessive toxicity. Therefore, the tumor results at the high doses in the mouse, while not discounted, should be used with caution.

The EPA Draft Cancer Guidelines from 1996 and 1999 (ref. 25) state that excessive doses may be determined by factors such as: significant toxicity or perturbation of physiological function; reduction in body weight gain of greater than 10% over the lifespan of the animals; and significant increases in mortality from effects other than cancer. Using this reasoning, I feel that excessive cholinesterase inhibition can be considered to be either a perturbation of physiological function or toxicity depending on what else is happening to the animal. It should be noted that using cholinesterase inhibition—or any other endpoint—when establishing that doses are excessive, does not imply an effect (either positive or negative) by that endpoint on the tumor response. It indicates a compromised animal where homeostasis is altered—"... that would confound the interpretation of study results to humans." Whether the response indicates an adequate dose or an excessive dose depends on the magnitude of the response. Although the CARC evaluates the adequacy of dosing on a study by study and often on a dose by dose bases, it strives for consistency across studies and chemicals. The CARC does not—as presented in Dr. Dementi's item 9 summary—"discount a dose level in question as excessive, and in so doing discount remarkable tumorigenic findings observed at that dose level." I feel the CARC adequately considered the issue of what constitutes excessive toxicity.

10) Acceptability of OSTP's (1985) definition of carcinogen, and if considered acceptable, the rigor of its application in CARC's interpretation of the malathion studies. (ref. 5, 8, 9, 11)

Dementi Summary: "The OSTP (White House Office of Science and Technology Policy) definition reads as follows: 'A chemical carcinogen may be a substance which either significantly increases the incidence of cancer in animals or humans or significantly decreases the time it takes a naturally occurring (spontaneous) tumor to develop relative to an appropriate background or control group. Either phenomenon is said to represent the effects of a carcinogen.' (pp. 10414-10415) I have sought from CARC its views as to the veracity of this definition of a carcinogen, but my question has not been acknowledged or addressed. I posed the question because it seemed to me that on certain of the tumorigenic end points, the committee appeared too focused on the first element of the definition (strict statistical treatment of tumor incidence) to the neglect of second element (rate of tumor development). Evidence of enhanced tumor development, including such findings as greater proportions of malignant versus benign tumors, tumor multiplicity, tumor size, decreased tumor latency, etc, may not yield statistical evidence of carcinogenicity, but yet constitute positive evidence of carcinogenicity according to the OSTP definition. If CARC owns this definition, then it should provide more evidence of its utilization in the interpretation of the end points at hand."

Response:

I do not feel that the EPA cancer guidelines (ref. 25) are not in conflict with the OSTP definition. In the 1999 draft they state, "In, general, observation of tumor effects under different circumstances lends support to the significance of the findings for animal carcinogenicity. Significance is a function of the number of factors present and, for a factor such as malignancy, the severity of the observed pathology." The CARC does consider both tumor incidence and enhanced tumor development when evaluating the carcinogenic potential. These factors are considered, in conjunction with the remainder of the data base in the weight of evidence determination. HED has been instructed to use the EPA Draft Guidelines for Carcinogen Risk Assessment (ref. 25) and therefore, should provide evidence that it is following these guidelines, not the OSTP.

For malathion, if the Committee determined that a tumor had significant evidence of increased severity or decreased time for a spontaneous tumor to develop, it would have to take that into consideration in the weight of evidence. The guidelines specify that no one issue should be taken in isolation. In the case of MCL, **I am recommending that the Committee reconsider this issue** to determine whether there is supportable evidence for increased severity or decreased latency in the absence of increased incidence. In most cases, it is difficult to determine whether there is decreased latency since few studies have serial sacrifices.

11) Incorporation of tumorigenic findings or the absence (or reduced incidences) of the same, at doses considered excessive. (ref. 4, 9, 10, 17, 18)

Dementi Summary: "Questioned here is the use of tumorigenic findings, or the absence of the same, in a dose group considered excessive by CARC. A prime example is CARC's use of the top two dose groups (6000 ppm and 12,000 ppm), considered excessive doses by the Committee, for assessing tumorigenicity among male rats, in the combined chronic toxicity/carcinogenicity study in the rat. I contend as improper the discounting of tumorigenic findings of one type at a dose level considered excessive, while utilizing decreased tumorigenic findings of another type in these excessive dose groups to discount positive findings at lower doses considered by the committee to be acceptable. By contrast, I contend that accepting tumorigenic findings at excessive doses is more defensible than accepting as negative a study without findings at excessive doses."

Response:

The following response to Dr. Dementi's concern was taken from a memorandum written by William Burnam (ref. 23). This memorandum was written by Dr. Burnam in response to the OCT-28-1999 memorandum from Dr. Dementi (ref. 17). I have made minor spelling and editing changes to original version.

The problem of setting doses for cancer studies and judging the significance of tumors at excessive doses is one that the CARC and Agency Cancer Risk Assessment Guidelines have been trying to deal with for a long time. This is what our current draft Agency Guidelines (page 2-12, 2-13) state:

- Excessive high dose: If toxicity or mortality is excessive at the high dose, interpretation depends on the finding of tumors or not.

  (a) Studies that show tumor effects only at excessive doses may be compromised and may or may not carry weight, depending on the interpretation in the context of other study results and other lines of evidence. Results of such studies, however, are generally not considered suitable for dose-response extrapolation if it is determined that the mode(s) of action underlying the tumorigenic responses at high doses are not operative at lower doses.
  (b) Studies that show tumors at lower doses, even though the high dose is excessive and may be discounted, should be evaluated on their own merits.
  (c) If a study does not show an increase in tumor incidence at a toxic high dose and appropriately spaced lower doses are used without such toxicity or tumors, the study is generally judged as negative for carcinogenicity.

In the malathion example, the CARC has determined that based on dose and tumor response, the liver tumors in mice and rats indicate different interpretations.

In the mouse study, there were liver tumors at the two high doses in both males and females but no increase in liver tumors at the lower doses. The two higher doses were judged by the CARC to be excessive, while the lower doses, where no increases in tumors were seen, were adequate. It should be noted that even though, these two higher doses were excessive in terms of different types of cholinesterase inhibition seen, there were sufficient mice at risk (living long enough) to determine a carcinogenic effect for these tumors and that this carcinogenic effect was part of the CARC weight of evidence in its determination of a "likely" classification. Since there were no tumors at lower, non-excessive doses, the liver tumors in mice were not used for dose-response extrapolation.

In female rats, a statistically significant increase in liver adenomas and carcinomas was seen at the highest dose—a dose considered excessive by the CARC. However, in contrast to the mouse study, there was also statistical significance at the next to the highest dose and a biologically significant increase at the two low doses when compared to the control. None of these lower doses were considered excessive. The dose response information from

this rat study, spanning the doses from excessive to adequate, is the basis for the dose response extrapolation for human risk assessment and contribute heavily to the classification of malathion as "likely."

The treatment of other tumors in the rat uses the same rationale as the CARC did for liver tumors in mice and rats. The combined incidence of adenomas and carcinomas of the male thyroid follicular cell showed a significant trend but no increases by pari-wise analysis. Again, even though the two highest doses were considered excessive, there were sufficient rats at risk to be used in a carcinogenic analysis by Peto's Prevalence Test. The facts that there was only a trend for this combined follicular cell tumor in males was not a major contributor to the CARC's classification decision.

Likewise, with the c-cell thyroid tumor in male rats, there were sufficient rats at risk at the two highest—although excessive—doses, to be used in a statistical analysis. This Peto analysis indicated [a] statistically significant pair-wise increase in carcinomas only at the 500 ppm level. No increases in carcinomas were noted at the higher doses nor at any doses for the combined adenomas and carcinoma analysis.

In summary, I see no reason to change our analysis of the tumor data based on Dr. Dementi's October 28, 1999, comments and I believe that the CARC has been consistent in its rationale and analysis of the presence of tumors at excessive and at adequate doses."

The follow-up memorandum from Dr. Dementi to Dr. Burnam (ref. 18) does not provide additional factual arguments. I feel that the guidelines cited above provide for the use of scientific judgement regarding the use of tumor data at excessive doses. In the case of the malathion rat study, the CARC determined that tumor responses occurring only at excessively toxic doses were not appropriate for use in dose-response extrapolation. The data were however, considered to be supporting evidence of carcinogenicity—**and were not discarded**.

12) Application of general principles of competing toxicity and increased mortality in mitigating expression at excessive doses of a tumorigenic dose-response occurring at acceptable lower doses (no specific references given, used ref. 4, 7, 17)
Dementi Summary: "In my opinion, having cited authoritative sources, competing toxicity and increased mortality at excessive doses may diminish or even preclude tumorigenic responses identified at lower doses. Furthermore, in consideration of the potential for such compromises of tumor expression to occur at excessive doses, negative or diminished findings at such doses cannot be accepted as negative evidence of carcinogenicity. It is more acceptable, as I understand, to accept positive findings at excessive doses unless (according to EPA's draft Cancer Guidelines) it can be shown such tumorigenic responses resulted from toxicity as opposed to tumorigenicity of the test material. I am not satisfied CARC has made proper use of these concepts, specifically, in discounting certain tumorgenic findings at dose levels considered excessive without demonstrating these arose secondary to toxicity, while on the other hand accepting diminished tumorigenic responses at excessive doses as negative evidence. There have been no statements from CARC clarifying its philosophy.

"I must add that in the case of liver tumorigenic responses in female rats in the combined chronic toxicity/carcinogenicity study with malathion, I concur with CARC's interpretation at all doses, including that for the highest dose group, as being consistent with my understand of the principles at issue here, i. e., there is no evidence the tumorigenic response observed at the highest dose, the only dose level considered excessive in females, was due to anything other than the tumorigenicity of the test material."

Response:
While the CARC does acknowledge that competing toxicity may impact the expression of tumors, it is difficult to determine this as a cause without appropriate supporting and /or mechanistic data. The cancer guidelines (ref. 25) note that:

> "...(c) If a study does not show an increase in tumor incidence at a toxic high dose and appropriately spaced lower doses are used without such toxicity or tumors, the study is generally judged as negative for carcinogenicity."

I feel that this should apply to specific tumor types as well. The CARC can not make suppositions about the validity of a negative (or the lack of a) tumor response at excessive doses without solid scientific evidence. In most cases however, the CARC considers their decisions to be protective since any tumors that might be masked by competing toxicity are usually at excessively toxic doses. Therefore, I feel no additional evaluation of competing toxicity is warranted.

13) Acceptability of the rat combined chronic toxicity/carcinogenicity study to evaluate carcinogenic potential in male rats (no specific reference given, used ref. 8, 10, 17, 18)
Dementi Summary: "I have difficulty accepting CARC's decision concerning acceptability of the study as essentially a *negative* study in male rats, specifically the male F344 rat. Discounting the top two doses as excessive, and accepting the lower dose levels, in my opinion precludes testing at adequately high dose levels. The findings suggest the need for another dose group somewhere between the 500 and 6000 ppm dose groups. It may be the F344 rat is a poor model due to competing toxicity. On the other hand, if CARC accepted the study as demonstrating tumorigenic findings in males at the lower doses, perhaps that would be the end of it. Given the male rat assessment is thus confounded, greater reliance must be placed on findings in females, i.e., as carrying more weight than a single gender finding."

Response: (also see response to item 11)
The 6000 and 12,000 ppm dietary concentrations were both considered excessive for male rats based on mortality [total mortality 18/55(33%)**, 14/55(25%), 26/55(47%), 39/53(74%)**, 56/56(100%)** for controls to the high dose] and cholinesterase inhibition in all three compartments. In contrast, the 500 ppm dose group was considered adequate to evaluate carcinogenicity. Although not explicitly stated in the CARC Report, there was evidence of some toxicity: 1) a non-statistically, but probably biologically significant increase in mortality at this concentration; and 2) a decrease in plasma cholinesterase (29%, $p \leq 0.01$). **I recommend that this be included in the new CARC Report.** Therefore, it is considered to be appropriate to use the 500 ppm dose when evaluating this study for the carcinogenic potential of malathion in the male rat—without requiring any intermediate doses. In addition, for the reasons noted above, the CARC felt that requiring a new test with the male rats was not necessary–any additional information would not alter the cancer assessment and classification which is already, "likely human carcinogen." I feel that the data would have supported the 500 ppm dose as adequate in the hypothetical situation where it was the high dose in the study.

14) Adequacy of Q* method to address risks posed by low dose tumorigenic findings, e.g., liver tumors in the female rat at 100/50 ppm, in the absence of a NOEL (ref. 15, 16, 17)
Dementi Summary: "This is a philosophical question raised by me that has not been discussed at any of the CARC meetings as I recall. It is my concern that to the extent low dose tumorigenic findings occur at more elevated incidences than expected based on those incidences at much higher doses (e.g. the female rat liver tumor response), for whatever reason, such as a change of mechanism across the dose range, can the Q* calculation, employing all doses, be expected to address risks posed at the low dose level. I am concerned low dose findings in this assessment, close to those that minimally inhibit cholinesterase are of peculiar concern to the public health, and petition for additional expert comment on the utility of the Q* method to deal with this. This is more a gut feeling than one borne of any particular expertise or evidence I bring to the table. The Q* method has been used by CARC to address public health risks based on the female liver tumorigenic response."

Response (see last paragraph, page 2):
In my opinion, the CARC meetings and documents are not the appropriate forum for philosophical discussions. These are better deliberated in Agency workgroups. The above philosophical question is generic and does not apply specifically to malathion. Therefore, my response is general and does not address malathion specifically. The CARC is required to follow the EPA Cancer Guidelines. The 1986 guidelines and 1996 and 1998 drafts are fairly specific regarding when linear extrapolation is to be deviated from. In order to use some other form of modeling, there has to be support from mechanistic data. The 1986 guidelines use the Q_1* (the linearized multistage procedure), while the new draft guidelines (1996 and 1999) discuss extrapolation from an LED_{10} or ED_{10}. Due to the fact these are still draft, and the method of quantitative risk assessment is still undergoing modification and clarification, HED has continued to use the Q_1* when linear extrapolations are warranted. Both the Q_1* and LED_{10} or ED_{10} methods are similar types of low dose linear extrapolation models which use all of the data at the high and low doses.

Reference 21 - Items identified by Dr. Dementi as either incorrect or inconsistent.

1) Dr. Dementi's comments concerning CARC Report p. vi, paragraph 3:
(see last paragraph, page 2 of this memorandum)

- The final CARC Report already was corrected to say "two tumors per dose level," therefore no follow-up action is needed.

- In the same paragraph, Dr. Dementi expressed concern about the use of the expression "mainly adenomas" when referring to the liver tumor increase in females.

  I feel that this concern may be valid since there are as many carcinomas at each dose as adenomas, with the exception of the 6000 ppm dose. This paragraph will be modified to say, "There was no statistical significance for carcinomas." In addition, the expression, "(an adenoma and carcinoma)" will be added to the comment that there were two tumors at each of the two low doses.

2) Dr Dementi identified an error in the date given for the Original Pathology Report in the table 1 title. This report was completed in 1994 while the table listed the date as 1997—the date that the statistics in the table were completed.

The table title will be modified to read: "Based on the Original 1994 Pathology Report ...."

3) Dr. Dementi expressed concern that there are inconsistencies between the methods for evaluating male rat follicular cell and c-cell tumors of the thyroid.

These two tumor types are discussed in the *CARC Responses to Issues Raised By Dr. Brian Dementi Regarding the HED Cancer Assessment of Malathion*, items 2 and 3 above.

4) Dr. Dementi noted that there appeared to be a discrepancy between the way the nasal and oral rat tumors were evaluated.

I discussed this concern in items 6 and 7 above, where I recommended that the oral tumor response be reevaluated by the CARC for the reasons that Dr. Dementi enumerated.

REFERENCES

from Brian Dementi:

1 Dementi, B.(1997). *Memorandum:* Brian Dementi, Toxicology Branch 1 to William Burnam, Chairman, Cancer Assessment Review Committee, dated **November 26, 1997.**

2 Dementi, B.(1998). *Memorandum:* Brian Dementi, Toxicology Branch 1 to William Burnam, Chairman, Cancer Assessment Review Committee, dated **February 23, 1998.**

3 Dementi, B.(1998). *Memorandum:* Brian Dementi, Toxicology Branch 1 to William Burnam, Chairman, Cancer Assessment Review Committee, dated **April 9, 1998.**

4 Dementi, B.(1998). *Memorandum:* Brian Dementi, Toxicology Branch 1 to Jerry Hardesty, dated **May 4, 1998.**

5 Dementi, B.(1998). *Memorandum:* Brian Dementi, Toxicology Branch 1 to William Burnam, Chairman, Cancer Assessment Review Committee, dated **May 29, 1998.**

6 Dementi, B.(1999). *Memorandum:* Brian Dementi, Toxicology Branch 1 to Sanju Diwan, Executive Secretary, Cancer Assessment Review Committee, dated **February 11, 1999.**

7 Dementi, B.(1999). *Recommendation to CARC Members* from Brian Dementi, Toxicology Branch 1, dated **February 24, 1999.**

8 Dementi, B.(1999). *Memorandum:* Brian Dementi, Toxicology Branch 1to Jess Rowland, Executive Secretary, Cancer Assessment Review Committee, dated **April 1, 1999.**

9 Dementi, B.(1999). *Memorandum:* Brian Dementi, Toxicology Branch 1 to William Burnam, Chairman, Cancer Assessment Review Committee, dated **April 27, 1999.**

10 Dementi, B.(1999). Addendum to Malathion Qualitative Risk Assessment Based on Fischer 344 Rat Dietary Study. *Memorandum*: Brian Dementi, Toxicology Branch 1 to William Burnam, Chairman, Cancer Assessment Review Committee, dated **May 18, 1999.**

11 Dementi, B.(1999). Malathion Combined Chronic Toxicity/Carcinogenicity Study in the F344 Rat (MRID No. 43942901). *Memorandum*: Brian Dementi, Toxicology Branch 1 to William Burnam, Chairman, Cancer Assessment Review Committee, dated **June 7, 1999.**

12 Dementi, B.(1999). *Memorandum:* Brian Dementi, Toxicology Branch 1 to William Burnam, Chairman, Cancer Assessment Review Committee, dated **June 21, 1999.**

13 Dementi, B.(1999). *Memorandum:* Brian Dementi, Toxicology Branch 1 to William Burnam, Chairman, Cancer Assessment Review Committee, dated **July 13, 1999.**

14 Dementi, B.(1999). *Memorandum:* Brian Dementi, Toxicology Branch 1 to William Burnam, Chairman, Cancer Assessment Review Committee, dated **July 22, 1999.**

15 Dementi, B.(1999). *Memorandum:* Brian Dementi, Toxicology Branch 1 to William Burnam, Chairman, Cancer Assessment Review Committee, dated **September 21, 1999.**

16 Dementi, B.(1999). *Memorandum:* Comments on September 20, 199 Draft CARC Report on Malathion. Brain Dementi, Toxicology Branch 1 to Jess. Rowland, Executive Secretary, Cancer Assessment Review Committee, dated **October 6, 1999.**

17 Dementi, B.(1999). *Memorandum:* Brian Dementi, Toxicology Branch 1 to Jess Rowland, Executive Secretary, Cancer Assessment Review Committee, dated **October 28, 1999.**

18 Dementi, B.(1999). *Memorandum:* Brian Dementi, Toxicology Branch 1 to William Burnam, Chairman, Cancer Assessment Review Committee, dated **November 12, 1999.**

19 Dementi, B.(1999). *Memorandum:* Brian Dementi, Toxicology Branch 1 to William Burnam, Chairman, Cancer Assessment Review Committee, dated **December 7, 1999.**

20 Dementi, B.(2000). *Memorandum:* Brian Dementi, Toxicology Branch 1 to William Burnam, Chairman, Cancer Assessment Review Committee, dated **January 12, 2000.**

21 Dementi, B.(2000). *Memorandum:* Brian Dementi, Toxicology Branch 1 to Jess Rowland, Executive Secretary, Cancer Assessment Review Committee, dated **February 7, 2000**.

22 Dementi, B.(2000). *Memorandum:* Brian Dementi, Toxicology Branch 1 to Jess Rowland, Executive Secretary, Cancer Assessment Review Committee, dated **February 9, 2000.**

Others:

23 - Burnam, W.(1999). *Memorandum:*"Tumors at Excessive Doses," William Burnam, Science Analysis Branch to members of the current CARC, dated **November 5, 1999.**

24 Caldwell, D.J. (1999). Review of Mononuclear Cell Leukemia in F-344 Rat Bioassays and Its Significance to Human Cancer Risk: A Case Study Using Alkyl Phthalates. *Reg. Tox. And Pharm.* 30:45-53.

25 EPA Guidelines for Carcinogen Risk Assessment, preliminary drafts, 1996, 1999.

26 The FIFRA Scientific Advisory Panel report, "A Set of Scientific Issues Being Considered by the a Agency in Connection with DDVP (Dichlorvos) Risk Issues," meeting date July 30, 1998.

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Chemical:	**Malathion**
PC Code:	**057701**
HED File Code	**21200 CARC**
Memo Date:	**03/30/2000**
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